How do flooding regime and traits related to oxygen uptake and transport affect plant performance?

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Chapter 3

Oxygen absorption by adventitious roots promotes the survival of completely submerged terrestrial plants

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Abstract

Flooding imposes stress upon terrestrial plants because it results in oxygen deficiency, which is considered as a major problem for submerged plants. A common response of terrestrial plants to flooding is the formation of aquatic adventitious roots. Previous research has shown that adventitious roots on submerged plants are capable of absorbing water and nutrients. However, there is no experimental evidence for the possible oxygen uptake function of adventitious roots or for how important this function might be for the survival of plants during prolonged submergence. This study aims to investigate whether adventitious roots absorb oxygen from water column, and whether this new function is beneficial to the survival of completely submerged plants. Taking *Alternanthera philoxeroides* (Mart.) Griseb. as a representative species, we conducted the profiling of underwater oxygen gradient towards living and dead adventitious roots on completely submerged plants, measured the oxygen concentration in stem nodes with and without adventitious roots, and investigated the growth, survival, and nonstructural carbohydrate content of completely submerged plants with and without adventitious roots. O_2 profiles in the water column of adventitious roots showed that adventitious roots absorbed oxygen from water. It is found that oxygen concentration in stem nodes having adventitious roots was higher than that in stem nodes without adventitious roots, which implies that the oxygen absorbed by adventitious roots from water was subsequently transported from the roots to other plant tissues. Compared to adventitious-root-pruned plants, adventitious-root-unpruned plants had slower leaf shedding, slower plant mass reduction, more efficient carbohydrate economy and prolonged survival when completely submerged. The adventitious roots of *A. philoxeroides* formed upon submergence can absorb O_2 from ambient water, thereby alleviating the adverse effects of oxygen deficiency, enabling efficient utilization of carbohydrates and delaying the death of completely submerged plants.

Introduction

Flooding profoundly affects a wide range of ecosystems, from river forelands to farmlands, and it is one of the main sources of natural disasters worldwide (Smith, 2013). Moreover, as a consequence of climate change, the frequencies and intensities of floods are expected to increase in the future (Hirabayashi *et al.*, 2013; Stocker *et al.*, 2013). Flooding is detrimental to many terrestrial plants as it generally hampers their growth and may ultimately cause their death (Voesenek *et al.*, 2006; van Bodegom *et al.*, 2008). However, some terrestrial plant clades have evolved a number of adaptive mechanisms that are considered to reduce the negative effects of submergence (Mommer & Visser, 2005). Such adaptations may constitute an important source of plant diversity in areas that have had a flooding regime for a long time and where adapted species have had the time to establish (Silvertown *et al.*, 1999).

The adaptations to flooding generally relate to coping with oxygen limitations given that, when plants become submerged, oxygen is an important limiting resource for metabolic performance and survival (Vartapetian & Jackson, 1997). The sources of O_2 potentially available to plants when completely submerged are internal, i.e. from their own photosynthesis, or external from the water column. Light availability is an important factor for photosynthesis; compared with the conditions above water, aquatic environments are generally considered to be shaded environments, since light is attenuated by surface reflection, back-scattering, and absorption by water and suspended particles (Sand-Jensen, 1989). This particularly applies to river water, in which the load of suspended sediment is often very high, and thus light transmission is poor. For example, median transmission in floodplains of the river Rhine is < 1% when flooded to 1 m depth, even at the lowest levels of suspended loads observed during flooding (Vervuren *et al.*, 2003). Similarly, seasonally flooded rice fields may also suffer from turbid conditions, with light penetrating <0.4 m deep into floodwater (Ram *et al.*, 2002). Such low light conditions result in a particularly unfavorable environment for photosynthesis. Additionally, photosynthesis will not only be limited by light during flooding, but also the availability of carbon dioxide is severely limited (Maberly & Madsen, 1998; Sand–Jensen & Frost–Christensen, 1999), as the slow diffusion rate in water will greatly hamper uptake rates by the leaves compared with those in air (Bowes, 1987; Madsen & Sand-Jensen, 1994). Boundary layers

around the leaves and stem are likely to be several orders greater in water than in air, particularly in stagnant or slow flowing water (Smith & Walker, 1980). These factors together indicate that, at these low photosynthesis rates, the oxygen dissolved in water becomes the main oxygen source for completely submerged plants, and plants must take up oxygen through their submerged tissues.

The supply of O_2 to plants in the water column diminishes because water contains less gas than the atmosphere, as diffusion of dissolved gases in water is 10000-fold slower than that in air (Jackson, 1985). Oxygen deficiency inhibits respiration causing an 'energy crisis´ in anoxic cells or tissues and ultimately results in the death of plants (Crawford & Braendle, 1996). Plants submerged due to the low availability of internal O_2, usually switch from aerobic to anaerobic respiration for energy conservation (Perata & Alpi, 1993). The level of carbohydrate storage in plant tissues greatly affects the survival during submergence. The low internal O_2 concentration induces more consumption of stored carbohydrates, accelerates the process of cell death and leads to low survival rates (Bailey-Serres & Chang, 2005).

Some terrestrial plants have evolved particular adaptive mechanisms to reduce the negative effects of submergence via low oxygen availability. For instance, leaves with gas films are able to improve their gas exchange with the water column and improve the internal aeration during complete submergence, because the gas films on submerged leaves enlarge the water-gas interface and also facilitate the uptake of oxygen via stomata, thereby bypassing the resistance of foliar cuticles (Pedersen *et al.*, 2009; Verboven *et al.*, 2014; Winkel *et al.*, 2014). Also, when submerged, terrestrial plants may acclimate to submerged conditions by developing new leaves with thin blades and undeveloped cuticles, so as to reduce diffusion resistance and take up oxygen from the surrounding water column more efficiently (Mommer *et al.*, 2004; Colmer *et al.*, 2011).

In addition to forming these gas films and acclimated leaves, a more common response of most terrestrial plants to flooding is the formation of an aquatic adventitious root system (Jackson & Drew, 1984). Aquatic adventitious roots contain very thin or no cuticles and have a large surface area per unit root biomass because of the small root diameter and numerous root hairs. Some literature has shown that aquatic adventitious roots on submerged plants are capable of absorbing water and nutrients, as substitutes for sediment roots which become

dysfunctional or die upon submergence (Končalová, 1990; Polthanee & Changdee, 2008; Sauter, 2013). It has also been suggested that adventitious roots may absorb oxygen from the surrounding water column (Rich *et al.*, 2012). However, to our knowledge, there is no experimental evidence for this possible oxygen uptake function of aquatic adventitious roots; or for how important this function might be for the survival of plants during prolonged submergence. Here we hypothesize that adventitious root uptake is an important O_2 source for plant survival under completely submerged and dark conditions.

To test our hypothesis, we applied a new methodology of experimentally pruning adventitious roots of submerged terrestrial plants and measured its impacts on oxygen uptake and on plant survival. We used *Alternanthera philoxeroides* (Mart.) Griseb., a submergence-tolerant terrestrial species as a model, as it is known to *de novo* produce a number of adventitious roots on the stem nodes in a short time upon submergence (Gao *et al.*, 2008). We tested our hypothesis in three steps. First, we monitored the oxygen uptake by adventitious roots during submergence in the dark; second, we measured the oxygen concentration in stem nodes with adventitious roots *versus* in stem nodes with all adventitious roots removed; third, we quantified the survival status of plants with or without adventitious roots (due to pruning treatment) during prolonged complete submergence in the dark.

Materials and methods

Species and plant preparation

Alternanthera philoxeroides（Mart.）Griseb., a perennial terrestrial plant of the Amaranthaceae family, originates in South America, but has spread to many parts of the world and is considered an invasive species in Australia, China, New Zealand, Thailand and the United States. This species possesses a good tolerance to submergence, upon which it usually produces adventitious roots on the nodes of the submerged stem. Under normal conditions, *A. philoxeroides* grows to a height of 50-120 cm, with a long, single or sparsely branched stem.

The *A. philoxeroides* plants used in the experiment were cultivated from cuttings that had been obtained from plants naturally growing on banks of Jialing River in the subtropical Chongqing District, southwest of China (29°49′N, 106°25′E).

Unbranched plants with stem length of ca. 35 cm were selected and cut at the stem base. Each cutting was planted in a 15cm diameter and 15cm deep plastic pot containing a soil mixture of 40% clay, 40% humus soil and 20% sand, two stem nodes of the cutting were buried in the soil for rooting. All these plantlets were kept in an open field of the experimental garden of the Key Laboratory of Three Gorges Reservoir Region at Southwest University, Chongqing (i.e. close to the collecting site), and cultivated under the same conditions of temperature (around 10-15 $^{\circ}$C in day-time), relative humidity (75-85%), light (daily max. 600 – 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and water provision (around 80% - 90% of soil water-holding capacity). After growing for about one month, all plants had rooted and were in good health, around 40 cm tall, ready for the submergence experiments.

Experimental set-up

Before complete submergence, 24 plants were harvested as the initial harvest group for measurement of the total leaf number, length and width of each leaf, leaf area and stem diameter of each plant. The dry mass of leaves, stem, rhizome, roots and adventitious roots were also quantified. Regression equations between the width, length and dry mass of leaves, and between the volume and dry mass of the stem were constructed; these equations were used to estimate the initial dry mass of the remaining plants (for analysis of relative growth rate; see below) before complete submergence. In this study leaf area was measured by a Leaf Image Analysis System (WD3 WinDIAS, Delta-T), and dry mass of each tissue was determined by weighing after oven-drying at 60°C for 72h. For the complete submergence experiment, 44 rooted plants were used, of which 24 plants comprised the control group with adventitious roots intact and 20 plants constituted the pruning group whose adventitious roots were to be pruned (see below). These potted plants were subsequently placed in 1.5 × 0.8 × 1.0 m (length, width, depth) water tanks, which were placed in a growth room, and submerged by adding tap water to the tank until the water level was 10 cm above the top of the stems. During the experiment, all plants were completely submerged, in complete darkness and the water temperature was kept at around 23-25°C. In order to maintain continuous oxygen availability, the water column was gently bubbled with air during the submergence treatment. Adventitious roots emerged from the stem nodes after 7-8 days of submergence. The control plants were kept intact, but once the plants of the pruning group started to develop any adventitious roots on

the nodes, these adventitious roots were carefully pruned from the attachment point to the stem under water with a small knife preventing any visual damage on the stem and leaves. After 15 days of complete submergence, 10 plants (seven plants with adventitious roots and three with adventitious roots removed) were chosen for O_2 measurements, and the others were still kept in the tanks for survival status testing (see below).

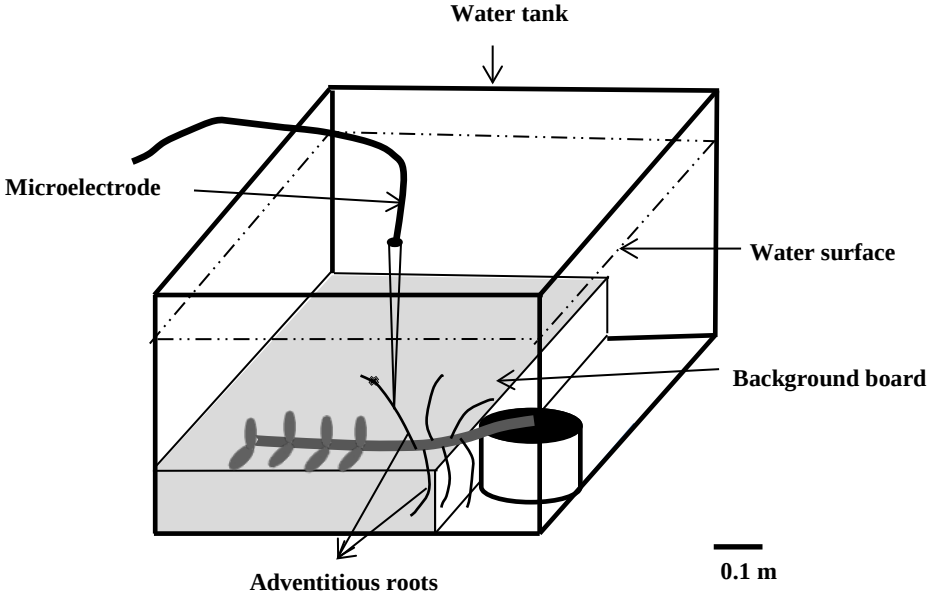

Figure 1. Schematic representation of experimental set-up for profiling of oxygen gradient towards an adventitious root surface under water. The plant with adventitious roots was placed at the bottom of the water tank and the target adventitious root was fixed onto the background board. The whole potted *Alternanthera philoxeroides* plant was submerged with air-saturated water for about 1 hour. The microelectrode was fixed on a micromanipulator, and used to measure the underwater oxygen profile over a distance of ca. 1200 µm by advancing a step of 50 µm every 5 seconds, moving towards the root surface.

O_2 measurements

A. Profiling of oxygen gradient towards submerged adventitious roots under water

To clarify whether adventitious roots on submerged plants are able to take up oxygen from water, we investigated underwater profiling of O_2 gradient towards.

(a) living adventitious roots, (b) dead adventitious roots (as a control for adventitious roots in the absence of metabolic activity) and (c) background (namely, no adventitious roots present).

(a) Living adventitious roots: Each selected completely submerged plant (in pot) with adventitious roots was transferred under water to a PVC tank (length × width × height: 0.8m × 0.5m × 0.4m) filled with air-saturated tap water, and stem, leaves and adventitious roots were fixed onto a background board (smooth foam board) which was positioned at the bottom of the tank (Fig. 1). One adventitious root below the foliated stem part, 10 -12 cm in length and 0.3 - 0.5 cm in diameter, was randomly chosen to conduct oxygen profiling and was fixed onto the background board far from the stem and other adventitious roots, thus preventing them from disturbing the oxygen gradient in water column. The water level of the air-saturated tap water in the tank was maintained at ca. 15 cm above the background board. A microelectrode (tip diameter = 10 µm, OX10, Unisense) mounted on a motor-driven micromanipulator controlled by microprofiling software (SensorTrace Pro, Unisense, Denmark) was used to determine the O_2 concentration profiles. These profiles were taken at a position of the adventitious root which was approximately 10mm away from the attachment point of the root to the stem, by advancing the electrode vertically downward over a distance of ca.1200 µm in steps of 50 µm every 5 sec, moving towards the adventitious root surface. The signal from the microelectrode was amplified using a picoamperometer with an analogue / digital converter. Electrode signals were converted into O_2 concentration using calibration between 0 and 100% of air equilibrium. The transfer of plants and microelectrode positioning were done in green light, all measurements were performed in darkness at 25°C. Four plants were used for oxygen profiling, and for each of these plants, one adventitious root was chosen for the profiling.

(b) Dead adventitious roots: When the underwater oxygen profiling of the living adventitious root of each of four plants was finished in procedure (a), the water in the tank was drained to a suitable water level so as to only de-submerge the target living adventitious root but leave the whole plant and other adventitious roots still submerged, the target living adventitious root was detached from the background board and then treated with hot steam for 2 minutes. This was sufficient to kill the living adventitious root and stop metabolic activities but did not affect the stem attached. Steaming treatment was done by boiling de-ionized water and conveying the steam through a thin glass pipette to the adventitious root. We checked the effectiveness of this method to kill the adventitious roots in pilot experiments, in which we used Evans blue to stain dead adventitious roots. After the steaming treatment, the dead adventitious root was fixed onto the background board again and the tank was refilled with air-saturated tap water to the same water level, the profiling of oxygen gradient towards the dead root under water was investigated in the same way as described in procedure (a).

(c) Background board: In order to check and preclude any artefacts caused by the background board on the O_2 profiles of water column submerging adventitious roots, O_2 profiles of water column above the background board without any plant tissues was investigated in the same way as described in procedure (a). Four replicates were measured.

(B) Oxygen concentration in submerged nodes

Node tissue O_2 concentration of *Alternanthera philoxeroides* plants with adventitious roots intact versus with adventitious roots removed was measured under the condition of complete submergence in darkness. Each potted plant was placed in the tank with a background board (Fig. 1), plants were mounted onto the background board and submerged 15 cm deep in air-saturated tap water. A 25 μm tip diameter O_2 microelectrode (OX25, Unisense) was mounted on a motor-driven micromanipulator and inserted 300 μm deep into the node. Three replicate plants with and without adventitious roots were measured, respectively.

Survival status testing

During submergence, the number of shed leaves was recorded every day as an indicator of decline in plant vitality. The complete submergence experiment lasted

for 38 days after which all plants in the tanks were harvested. The total leaf number, leaf area, stem diameter, and dry mass of each tissue of each plant were measured in the same ways as for the initial harvest. The firmness of the stem tip tissues of each plant was recorded as an another measure of its vitality, where we interpreted a firm tip as belonging to a still surviving plant and a soft tip to a dying plant. Also, the nonstructural carbohydrates (NSC), including both sugars and starch, in stem and leaves of final harvested plants were analyzed as a measure of anaerobic *versus* aerobic respiration (see Introduction). For methodological details on the NSC assay see Wong (1979) and Hoch *et al*. (2003).

Data analysis

The relative growth rate (RGR) of dry mass of each individual plant was calculated as:

$$RGR\ (g \cdot g^{-1} \cdot day^{-1}) = (\ln A_2 - \ln A_1) / (t_2 - t_1)$$

where A_1 is the estimated dry mass of each plant before complete submergence. A_1 was the sum of the estimated dry mass of leaves, stem, rhizome, roots and adventitious roots, in which initial dry mass of leaves was estimated from regression equations using leaf length and width, and initial dry mass of stem was from regression equations using stem volume (see above); the initial dry mass of roots, rhizome and adventitious roots were the mean values got from the initial harvest group (see the Experimental set-up). A_2 was obtained from the final harvest of each plant after complete submergence, while t_1 and t_2 represent the start and end date of complete submergence, respectively.

Leaf area ratio at final harvest was determined as: LAR = total leaf area / total plant dry mass.

A repeated measure ANOVA was run for testing the differences of oxygen profile between living adventitious roots, dead adventitious roots and background board without adventitious roots over the interval of 600 µm to 0 µm from the adventitious root surface. A Chi-square test was run for testing the difference in numbers of plants with firm versus soft apical tissues. An independent T-test was carried out to test for differences in oxygen content in nodes, final recorded leaf number, LAR and RGR between control and pruned plants. In addition, a repeated

measure ANOVA was run to determine the differences in dry mass with treatment as between-subjects factor and tissue as within-subject factor, also an independent T-test was carried out to test the differences between treatments in dry mass of each tissue. Data transformation was performed to equalize variances when necessary in repeated measure ANOVA, significant differences were reported at $P < 0.05$. All analyses were performed using SPSS 21.

Results

It was found in the study that oxygen profiles in the water column differed between experimental treatments, i.e. background board, living adventitious roots and dead adventitious roots. In this study, it appeared that 600 μm was the distance range within which the dissolved oxygen concentration in water column was affected by the presence of living adventitious roots (Fig. 2). From a distance of 600 μm to 0 μm, oxygen profiles in water column adjacent to living adventitious roots were significantly different from that adjacent to background board and dead adventitious roots, oxygen profiles in water column adjacent to living adventitious roots had a smaller slope than that in water column adjacent to dead adventitious roots (Fig. 2). Furthermore, the oxygen concentration in water at the surface (at distance 0 μm) of living adventitious roots was lower than that of dead adventitious roots. In this study, we also investigated the oxygen concentration in stem nodes of completely submerged plants, we found that oxygen concentration in the stem nodes was significantly higher ($P = 0.034$) in the plants with adventitious roots than in those without adventitious roots (Fig. 3).

In the experiment, it was shown that in the first 14 days of complete submergence, no leaves shed from the plants either with or without adventitious roots (Fig. 4A). However, after 26 days of submergence, the plants started to lose some leaves, the plants without adventitious roots always lost more leaves compared with plants with adventitious roots. It is clear that the presence or absence of adventitious roots affected the tolerance of plants to complete submergence, at the end of study, the percentage of plants with soft stem apical tissues (Fig. 4B) was significantly larger in the adventitious root pruning group than that in the group with intact adventitious roots (Chi-square test, $P = 0.039$).

Under complete submergence in the dark, plants cannot carry out photosynthesis to accumulate carbohydrates, so plants can only consume carbohydrates already

stored in tissues before submergence, and consequently, the experimental plants generally had a negative relative growth rate (Fig. 5A). The plants without adventitious roots had significantly higher consumption of biomass, i.e. more

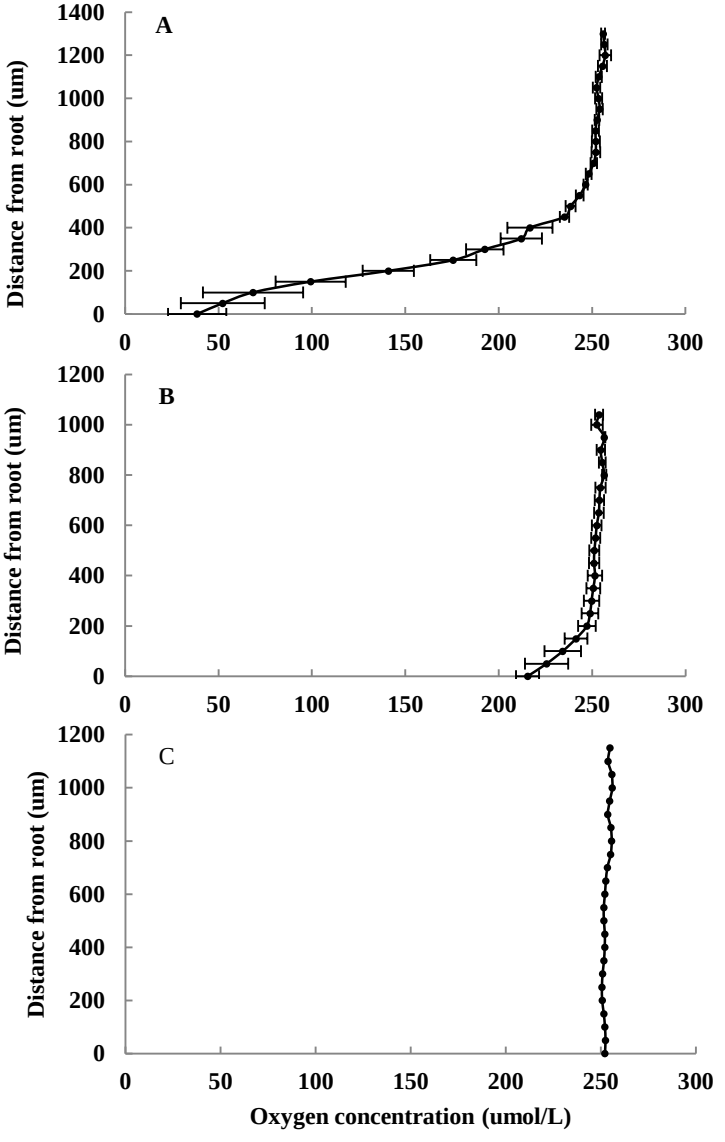

Figure 2. Oxygen profiles in water column adjacent to (A) living adventitious roots (n = 4), (B) dead adventitious roots (n = 3) and (C) background board without roots (n = 4) in darkness at 25°C. Values are given in mean ± SE. By using an O_2 microelectrode (tip diameter 10 μm), underwater profiling was conducted towards a point of the adventitious root, the point was approximately 10 mm away from the attachment point of the adventitious root to the stem of *Alternanthera philoxeroides*.

negative RGR, than plants with intact adventitious roots during the submergence (P = 0.041). Due to the slower leaf shedding, LAR of plants with adventitious roots was significantly higher than that of plants without adventitious roots at final harvest (P = 0.044) (Fig. 5B). It was revealed in this study that adventitious roots had impact on the consumption of carbohydrates. The concentration of leaf soluble sugars showed no significant difference between the two treatments (P = 0.421, Fig. 6), but the starch concentration of leaves in plants with adventitious roots was significantly higher than that in plants without adventitious roots (P = 0.015). The concentration of soluble sugars and starch in stems had the same trend as that in leaves, only the starch concentration of stem in plants with adventitious roots was significantly higher than that in plants without adventitious roots (P = 0.026).

In the study, we found that, after a period of complete submergence, stem mass took the largest proportion of the whole plant mass in all plants. In plants either with adventitious roots or without adventitious roots, the stem had higher mass than other organs (Fig. S1). Plants with intact adventitious roots had significantly higher stem mass and rhizome mass than plants with adventitious roots pruned. However, no significant differences were found between plants with or without adventitious roots in leaf and root mass (Fig. S1).

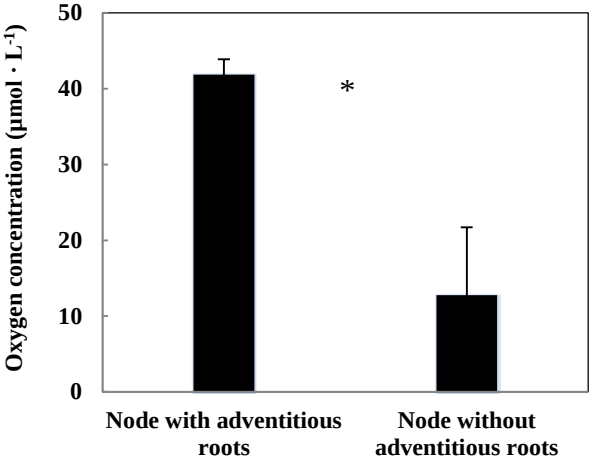

Figure 3. O_2 concentration (mean ± SE, n = 3) in stem nodes with adventitious roots or without adventitious roots in darkness at 25°C under complete submergence. During the measurement, *Alternanthera philoxeroides* plants were completely submerged to a depth of 25 cm. Asterisk indicates a significant difference at $P < 0.05$ between treatments (unpaired t - test).

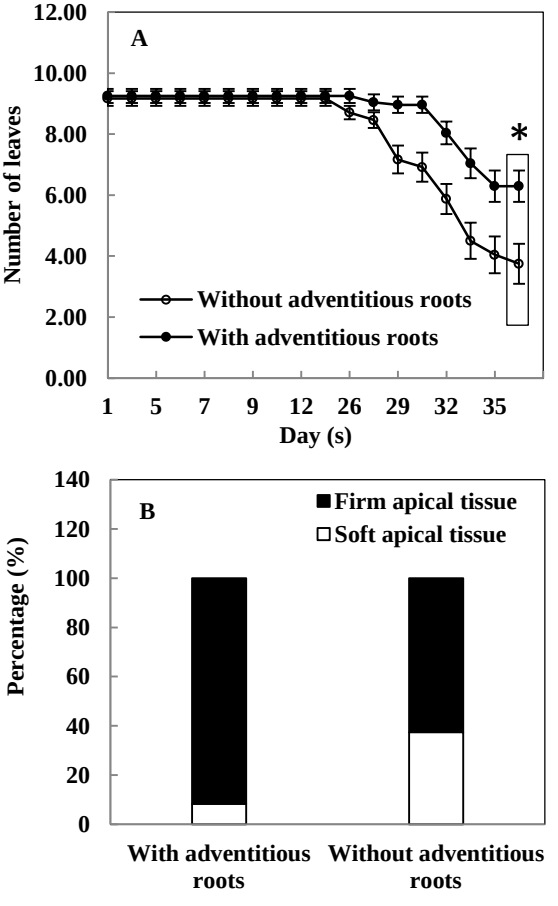

Figure 4. Indicators of plant vitality. Dynamics of leaf shedding (A) and stem apical tissue vitality (B) of *Alternanthera philoxeroides* plants with adventitious roots and without adventitious roots during the complete submergence in darkness at 25°C. In figure A, values are given in mean ± SE (n = 17) and asterisk indicates that the number of leaves on plants between the two treatments were significantly different ($P < 0.05$, unpaired t-test) on the last recording day. In figure B, the pruning of adventitious roots had negative effects on the stem apical tissue vitality of plants at final harvest. (Chi-square test, df = 1, $X^2 = 4.246$, $P = 0.039$, 17 plants used in each treatment).

Discussion

Oxygen uptake and transport through aquatic adventitious roots

A common response of plants to flooding is the formation of an adventitious root system, which usually forms on their stem or branches (Etherington, 1984; Naidoo & Naidoo, 1992; Rich *et al.*, 2008; Rich *et al.*, 2012). Aquatic adventitious roots

are adapted to the flooded environment and support or replace the primary soil roots, which usually die and lose their water and nutrient uptake functions during prolonged flooding (Sauter, 2013). Recent research revealed a new function of aquatic adventitious roots: production of internal oxygen and carbohydrate by photosynthesis in chloroplasts in root cells (Rich *et al.*, 2008; 2011). In our study, we have provided experimental evidence for another function of aquatic adventitious roots, i.e. uptake of external oxygen from the ambient water column. By comparing the oxygen profiles in the water column from the surface of living adventitious roots outwards with those of dead adventitious roots (Fig. 2), we found clear evidence that the living adventitious roots depleted the oxygen much more strongly and over a larger distance than dead ones, which resulted in shallower slope of the oxygen profile close to the living adventitious roots. The reason for this stronger oxygen depletion and resultant shallower slope is that, compared to the dead adventitious roots, the living adventitious roots had a bigger demand for oxygen, some amount of the oxygen were consumed by the respiration of living root cells, and other amount of the oxygen were transported out from the

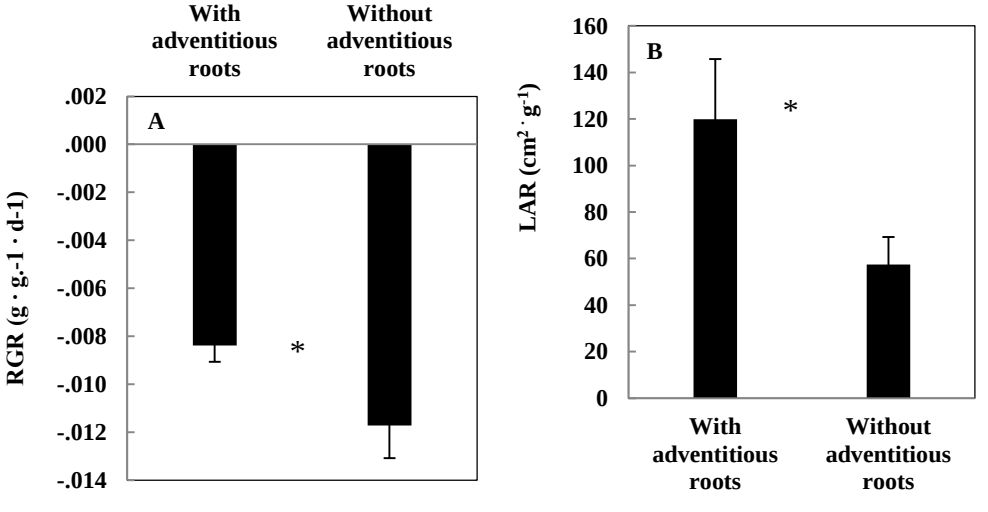

Figure 5. Relative growth rate (RGR) (means ± SE, n = 17) of dry mass (A) and leaf area ratio (LAR) (B) of *Alternanthera philoxeroides* plants with adventitious roots and without adventitious roots after 38 days complete submergence in darkness at 25°C. Asterisk indicates significant differences between the two treatments ($P < 0.05$, unpaired t-test).

adventitious roots to other parts of plants, accordingly, the living adventitious roots had a larger oxygen flux than dead adventitious roots. Strikingly, and consistent with the depletion outside the adventitious roots, the internal oxygen concentration of stem nodes with intact adventitious roots was considerably higher than those of stem nodes from which the adventitious roots had been cut off (Fig. 3). These findings show clearly that the oxygen uptake from the water column by adventitious roots can be transported to other part of plants. Uptake of oxygen from the water column into the plant has been observed for leaves (Mommer *et al.*, 2004), but to our knowledge, our study is the first time to show this process in aquatic adventitious roots.

Benefit of oxygen uptake by aquatic adventitious roots to submerged plants

When the completely submerged plants do not get internal oxygen from photosynthesis because of poor illumination due to turbid water or deep

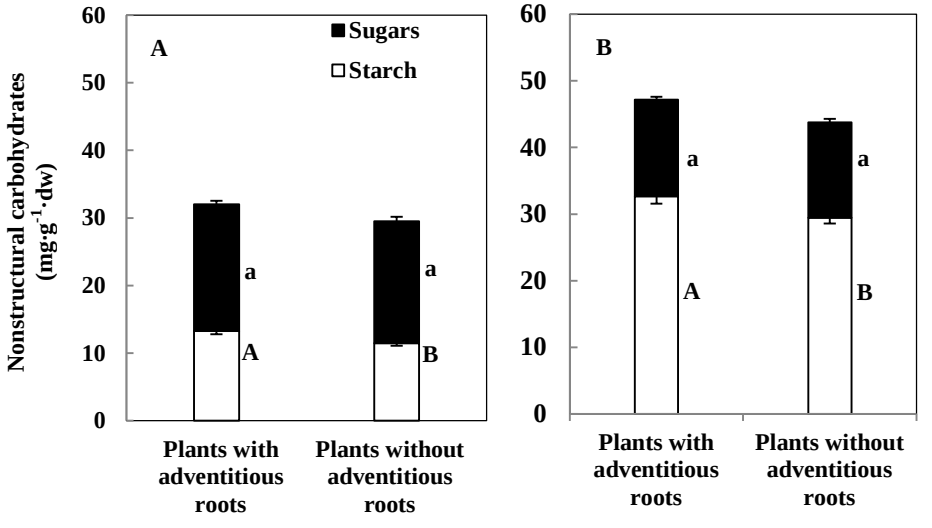

Figure 6. Content (means ± SE) of nonstructural carbohydrates (including starch and soluble sugars) in leaves (A) and stems (B) of completely submerged *Alternanthera philoxeroides* plants with adventitious roots and without adventitious roots. For either soluble sugars or starch, different letters indicate significant difference ($P < 0.05$) between treatments. Stem nonstructural carbohydrates content were determined with 20 replicates for plants with adventitious roots and without adventitious roots respectively, leaf nonstructural carbohydrates content were determined with 8 replicates for plants with adventitious roots and 6 replicates for plants without adventitious roots.

submergence, the water column functions as the only possible source of oxygen for the plant. Many studies have investigated that these floodplain species usually employ some adaptive responses to increase the gas exchange under water to overcome the negative effects of submergence, which include both metabolic and morphological plasticity, such as by enhanced elongation and reorientation of the leaves, by the increase of the amount of air space (aerenchyma) within the shoot and the roots, by switching to anaerobic metabolic pathways to prevent energy deficits (Perata & Alpi, 1993; Armstrong *et al.*, 1994; Blom, 1999; Colmer, 2003; Voesenek *et al.*, 2003). Furthermore, it has been hypothesized that gas films on leaves (Pedersen *et al.*, 2009) enable stomata to remain open; uptake of O_2 via stomata would bypass the resistance of cuticle and epidermal cells to O_2 entry into submerged leaves (Verboven *et al.*, 2014). Therefore, the gas films improve gas exchange under water, with benefits to whole-plant internal aeration and growth of completely submerged plants. However, not all flooded plant species can produce leaf gas films under water, so many or most species have to deal with the diffusion resistance to O_2. Some plant species can produce new acclimated leaves under water, with lower cuticle thickness and epidermal cell wall thickness promoting oxygen diffusion (Frost-Christensen *et al.*, 2003; Mommer *et al.*, 2004; Mommer & Visser, 2005). However, completely submerged plants take several weeks to produce new acclimated leaves under water, before that the adventitious roots may already have formed and played an important role in O_2 uptake from the water column. Moreover, adventitious roots may be the only important source of absorbing oxygen from ambient water for most flooded plant species, especially under deep submergence, where they probably do not grow any new acclimated leaves. Hence, the function of aquatic adventitious roots to absorb O_2 from the water column thereby improving the plant internal O_2 status may be especially important in dark conditions, such as in turbid water or under deep submergence. Further, even in more sunlit water these adventitious roots may also improve aeration of plants during the night.

There may be two more reasons why adventitious roots may be more effective at improving the oxygen supply to plants during prolonged submergence. Firstly, the ratio of total surface area to total volume of adventitious roots is higher than that of leaves. Moreover, some research have found that the percentage of aerenchyma in adventitious roots is larger than that in leaves of flooding tolerant plants (Visser *et al.*, 2000; Mommer *et al.*, 2005; Rich *et al.*, 2012), which means that

adventitious roots use less oxygen for cell respiration compared to leaves while they can transport relatively more O_2 to other plant parts. Secondly, compared to uptake through leaves, the oxygen taken up by adventitious roots can easily reach the sediment root system to improve their coping with the flooded conditions, due to the short distance from adventitious roots to soil roots (Sauter, 2013).

Submergence-tolerance enhancement of plants due to oxygen uptake by aquatic adventitious roots

Complete submergence imposes considerable stress on plant functioning, predominantly by way of oxygen deprivation. Low oxygen levels in plants limit aerobic respiration and other essential oxygen-dependent processes (Armstrong & Gaynard, 1976; Laan *et al.*, 1990). Anaerobic metabolic pathways, such as fermentation, may partly compensate the low ATP yield from impaired aerobic metabolism (Perata & Alpi, 1993; Gibbs & Greenway, 2003), but these pathways are far less efficient than aerobic respiration and thus reduce the pool of carbohydrate reserves relatively rapidly (Laan & Blom, 1990; Guglielminetti *et al.*, 1997). Submergence-induced oxygen deficiency in plants is therefore inevitably accompanied by carbohydrate and energy deficits. During a long period of submergence, the increasing consumption of carbohydrates, and possibly the accumulation of toxic substances in plants (e.g. sulfide, reactive oxygen species (ROS), Fe^{2+}, Mn^{2+}, etc.), result in loss of biomass and ultimately in death of many plant species. In our study, the starch in plants with adventitious roots was significantly higher than that in plants with adventitious roots experimentally removed when stopped complete submergence (Fig. S1.). Moreover, the rates of leaf shedding, loss of plant and stem biomass and decrease of LAR in plants with adventitious roots were lower than those in plants without adventitious roots; These experimental results support our expectation that O_2 absorbed by adventitious roots improves the O_2 availability in the plants, enabling them to use their carbohydrate reserves more efficiently and thereby enhancing their flooding tolerance. It is clearly shown in our study that oxygen uptake by adventitious roots prolonged plants' survival during complete submergence, which was indicated by a lower proportion of plants having soft apical tissues (a symptom of dying) in plant group with adventitious roots compared to the plant group without adventitious roots.

In summary, the aquatic adventitious roots of *A. philoxeroides* that develop during prolonged complete submergence can absorb O_2 from the surrounding water column, thereby alleviating the adverse effects of oxygen deficiency, enabling efficient consumption utilization of carbohydrate reserves and delaying the process of plant death. The next research challenge will be to study the occurrence and relative importance of plant O_2 uptake by aquatic adventitious roots in other plant species in different biomes of the world, and under different flooding regimes (e.g. partial submergence), and to explore how the O_2 uptake capacity of aquatic adventitious roots affects species' competitiveness and ecological adaptation to environments.

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Supplementary information

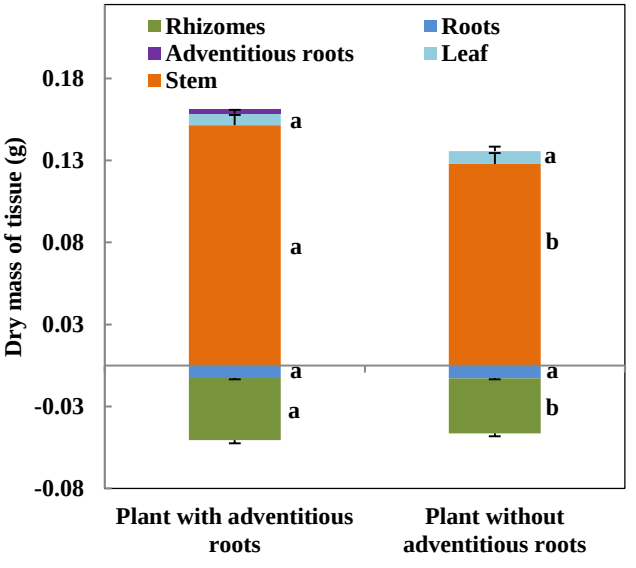

Figure S1. Organ dry mass (g) (mean ± SE, n = 17) of completely submerged *Alternanthera philoxeroides* plants with adventitious roots and without adventitious roots after 38 days of complete submergence in darkness at 25°C. For each plant organ, different letters indicate that organ dry mass differed significantly ($P < 0.05$) between treatments.